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Step change of co-ion, a new option in capillary zone electrophoresis

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ABSTRACT

A new variant of a dynamic step of the operational ionic matrix in capillary zone electrophoresis has been developed. The method extends the range of pK values of substances that can be analysed in one run and opens up a new means of optimization of the separation in comparison with classical zone electrophoretic mode where the composition of the background electrolyte–ionic matrix is maintained constant. The method is based on carrying out the separation at one pH for a certain period of time and, at another pH for another period. The change in the pH of the operational ionic matrix during the separation run is achieved by a fast-moving step of the co-ion. The method enables one to shorten the analysis time and is easy to use on commercial equipment. The analytical potential of the method is demonstrated by the separation of a six-component model mixture of phenol derivatives with 5 units difference in pK values.

INTRODUCTION

Electrophoretic separations of complex mixtures often do not yield a complete separation of all substances and/or the analysis time is too long if the separation run is carried out at a constant composition of the separation matrix (background electrolyte). Such cases can be expected especially when mixtures of substances covering a broad range of pK values are to be analysed.

Recently, a new option has been developed [1] for coping with this problem. The proposed method enables one to separate a sample in an on-line arrangement at two different pHs, which increases the separation power and provides more possibilities for optimization of the separation time. The basis of the technique is the generation of a dynamic change of the background electrolyte which proceeds fast along the separation capillary and introduces new operational conditions (pH).

These changes can be classified according to the direction of the migration and the shape of the concentration profile. In principle, with respect to the electromigration of solutes, there are two possibilities for dynamic changes, namely dynamic changes in the composition of the co-ion and/or the counter-ion. With respect to the shape of the dynamic change used, it has been shown that three principal types can be distinghuised, namely step, pulse and gradient.

In recent papers, the utilization of a pH step [2] and pulse [3,4] has been report-

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ed, with H^+ serving as the counter-ion. Further, the use of a dynamic pH gradient has been investigated in which H^+ served as the co-ion [5,6]. The common feature in these studies was direct control of the H^+ flow from the anodic chamber, which is easy as long as a reasonable pH range is used, *i.e.*, an acidic medium. There is, of course, a problem in controlling the H^+ flow at a concentration level of 10^{-7} ml/l, when a pH range from 7–11 should be used.

The objective of this paper is to show that the problem of dynamic buffering of pH in neutral and alkaline media may be easily solved by employing a dynamic step of the buffering anionic system. The technique proposed is exemplified by the separation of 2,5-dinitrophenol, 4-nitrophenol, 3-nitrophenol, 3-chlorophenol, 4-chlorophenol and phenol, were the analysis was started at pH 7 and finished at pH 11, and the appropriate dynamic change in pH was created by a moving boundary of carbonate → oxalate.

EXPERIMENTAL

A laboratory-assembled system was used in all electrophoretic experiments. The fused-silica capillary (VŠCHT, Prague, Czechoslovakia) was 58 cm × 85 μm I.D. Its inner surface was coated with linear polyacrylamide according to Hjertén [7].

A Jasco Model 875 UV detector was used (Japan, Spectroscopic, Tokyo, Japan), modified by replacing the original flow cuvette with a capillary holder. The detection cell was situated 42 cm from the injection end of the capillary (cathode side). The full-scale range was 0.02 absorbance units. The wavelength selected was 232 nm.

A laboratory-made high-voltage power supply was used and is described elsewhere [8]. The driving current was 40 μA at 12 kV across the whole capillary.

A Servogor (Goerz, Vienna, Austria) model 2S linear recorder was used. The sample was injected by a siphoning system: the sample contained a $3 \cdot 10^{-4}$ *M* solution of each solute and the sampling time was 5 s. The amount sampled was 10^{-13} mol at a 70-mm difference between levels of buffer and sample.

To prevent changes in the pH in the cathodic electrolyte chamber owing to electrolysis, it was filled with buffer. For pH 7.2, 8.3 and 9.5 N-(2-hydroxyethyl) morpholine, tris(hydroxymethyl)aminomethane and 2-amino-2-methyl-1,3-propanediol, respectively, were used.

All chemicals were of analytical reagent grade and were obtained from Lachema (Brno, Czechoslovakia), except 4-nitrophenol (Merck, Darmstadt, Germany), 3-nitrophenol (Loba, Vienna, Austria) and N-(2-hydroxyethyl)morpholine, tris(hydroxymethyl)aminomethane and 2-amino-2-methyl-1,3-propanediol (Serva, Heidelberg, Germany).

The procedure for producing the dynamic step of the co-ion (anion) was simple, as shown in Fig. 1. The injection end of the capillary together with a platinum wire serving as a cathode are dipped into the primary electrolyte chamber and subsequently into the modifying electrolyte chamber.

RESULTS AND DISCUSSION

For work at high pH, especially if the pH is changed during the run, it is very important either to eliminate electroosmosis or to keep the electroosmotic flow in the

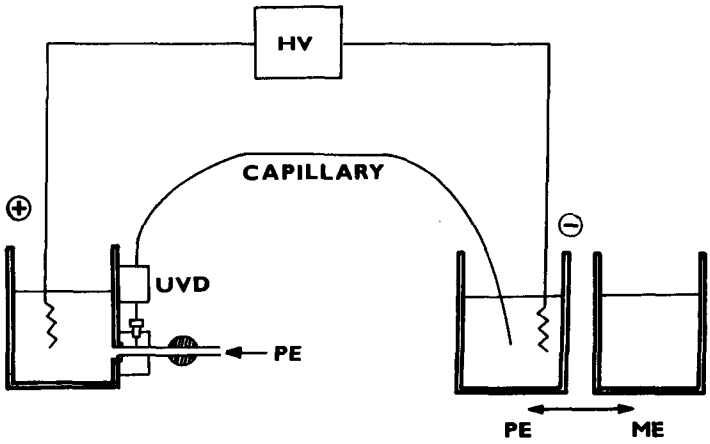

Fig. 1. Scheme of the electrophoretic equipment with modification of the ionic matrix. HV = High-voltage power supply; UVD = ultraviolet detector; PE and ME = primary and modifying electrolyte, respectively.

broad pH range as constant as possible, in order to ensure reproducibility of measurement. For this reason the inner surface of the capillary was coated with a layer of linear polyacrylamide and the electroosmotic properties of the coated capillary were tested by using picric acid, servingt as a mobility standard. By measuring the migration velocity of picric acid, the value $u(\text{eo}) \leqslant 4 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} for cathodic electroosmosis was found. This value was checked regularly and was stable during all experiments (about 1 month).

Nitro and chloro derivatives of phenol were selected as model species and are listed in Table I. Obviously, these species have very close ionic mobilities and they cover a broad range of pK values (5.2 − 10).

The separation of a model mixture of the above species at various constant pH values of the primary electrolyte are shown in Fig. 2. Fig. 2a shows the separation of pH 7.2 At this pH only the most acidic compounds, 2,5-dinitrophenol and 4-nitrophenol, can be analysed, as the other substances do not have sufficient mobility to reach the detector in an acceptable time. Analysis at pH 8.3 is shown in Fig. 2b. At this pH the species with increasing pK values up to 9.38 (4-chlorophenol) reach the

TABLE I

MOBILITIES AND DISSOCIATION CONSTANTS [9] FOR A MODEL MIXTURE

Substance	Mobility (10^{-9} m^2 V^{-1} s^{-1})	pK
2,5-Dinitrophenol	31.3	5.216
4-Nitrophenol	33.4	7.149
3-Nitrophenol	33.4	8.399
3-Chlorophenol	33.4	9.023
4-Chlorophenol	33.4	9.378
Phenol	34.4	9.998

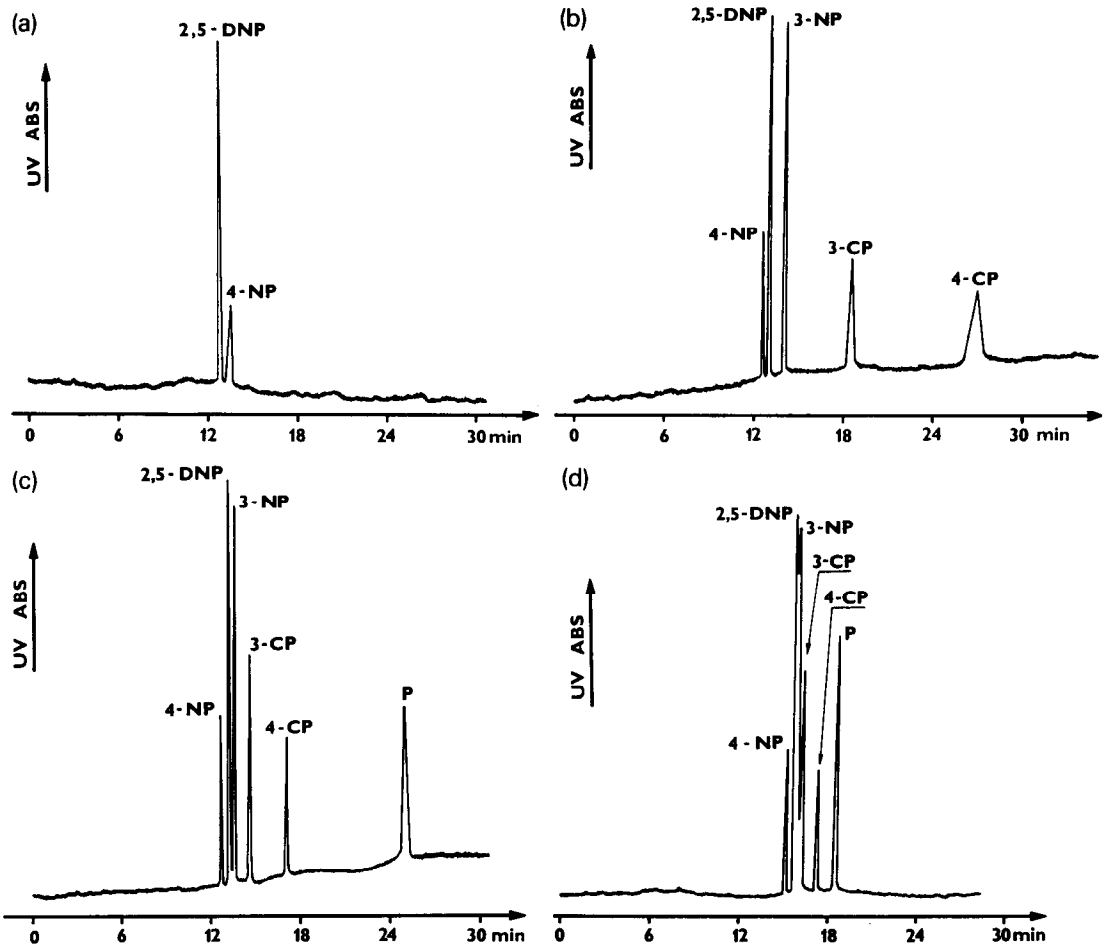

Fig. 2. Capillary zone electrophoresis of the model mixture in the primary electrolyte (0.01 *M* lithium oxalate) at constant pH: (a) 7.2; (b) 8.3; (c) 9.5; (d) 10.5. DNP = 2,5-dinitrophenol; 4-NP = 4-nitrophenol; 3-NP = 3-nitrophenol; 3-CP = 3-chlorophenol; 4-CP = 4-chlorophenol; P = phenol.

detector in less than 30 min. By increasing the pH to 9.4 and 10.5 (Fig. 2c and d), it is possible to analyse all the substances up to pK = 10 (phenol), but the 2,5-dinitrophenol–3-nitrophenol pair gives a mixed zone. The time required for a run is 25 and 19 min, respectively.

Obviously, one constant pH value during a run does not result in an acceptable analysis, and a dynamic step of pH may be used advantageously here.

The principle of the proposed method is depicted in Fig. 3, where the calculated trajectories (time *vs.* position in the capillary) of the separated substances are plotted. As can be seen in the lower part, all substances are easily separated in the primary ionic matrix, but the mobilities of 3-nitrophenol, 3-chlorophenol, 4-chlorophenol and phenol are too low to reach the detector in a reasonable time.

By replacing oxalate with carbonate, a modifying step is created, which in-

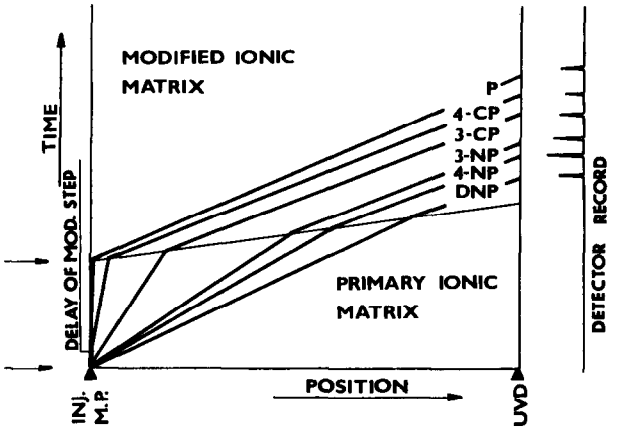

Fig. 3. Principle of the method. Trajectories of the sample components in the $x-t$ plane. INJ, injection; UVD, ultraviolet detection; M.P., starting–modification point. On the right the corresponding detection record is shown for abbreviations, see Fig. 2.

creases the pH along the column to 11. Hence, a new modified ionic matrix is created along the migration path. The dynamic step of carbonate → oxalate, *i.e.*, of modified → primary matrix, is faster than for any of the actual solutes and it passes them. When the solutes are in the modified matrix they are fully ionized and migrate with high velocities to the detector. Obviously, the separation can be affected by setting up pH values of the primary and modified matrix–electrolyte (in Fig. 3 we are changing the slopes of the trajectories), or we can change the time delay of the modifying step.

Experiments in which the dynamic step was utilized are shown in Fig. 4. In Fig.

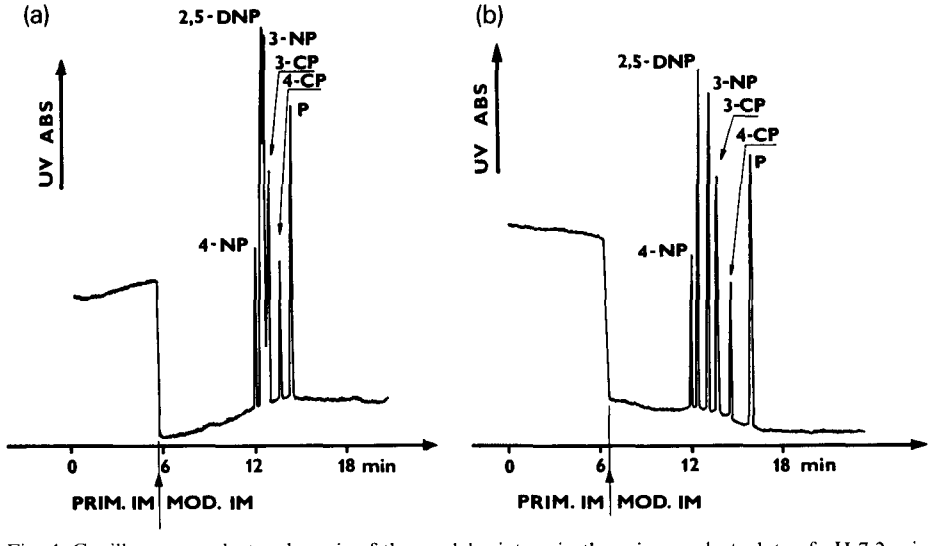

Fig. 4. Capillary zone electrophoresis of the model mixture in the primary electrolyte of pH 7.2 using ar ionic matrix step. Time delay of the step: (a) 0 s; (b) 20 s.

4a, the time delay of the step was 0 s, and analysis was performed in less than 13 min. The starting and final pH values were 7.2 and 11, respectively. Here, 2,4-dinitrophenol, 3-nitrophenol and 3-chlorophenol form the mixed zone. It is clear that the residence time of these substances in the primary matrix (pH 7.2) was too short. By introducing a time delay of 20 s, *i.e.*, by increasing the time of separation and residence time in the primary electrolyte, complete separation was achieved in 16 min (Fig. 4b). An additional increase in the time delay then leads only to an increased time of separation.

Finally, we conclude that a dynamic step of the co-ion provides a new means of increasing the separation power and optimizing the separation. The advantage of this method is that it requires a very simple mechanical experimental arrangement and may be easily used in commercial equipment.

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